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The Impact of Nicotine Accumulation Exposure on *Lithobates catebeianus* Larvae Mortality

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Introduction

- Cigarette butts are the most littered item in the world and contain more than 4,0000 chemicals, including the organic compound nicotine (Slaughter et al. 2011).
- These chemicals end up washing into lakes an streams, potentially harming wildlife.
- Amphibians, such as *L. catebeianus* or the American Bullfrog, act as indicator species due to their extreme sensitivity to environmental disturbances.
- Investigating the effects of introduced chemicals on indicator species provides insights into how it may impact the entire aquatic ecosystem.
- The purpose of this research project was to obtain data to help determine the impact nicotine accumulation has on as *L. catebeianus* larvae mortality.

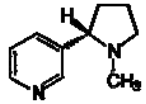

Fig. 1- Chemical formula for nicotine, an organic compound found in cigarettes.

Methods

- Seventy-eight *L. catebeianus* larvae, in similar developmental stages, were captured from Pioneers Park Nature center ponds where any tobacco usage is prohibited and 20 were sorted into each of 3 mesocosms containing 200 L of water.
- A bottle of nicotine solution containing 94 % vegetable glycerin (VG) and 6 % nicotine (1 mL = 60 mg of nicotine) and a bottle of 100% VG was used for the experiment.
- Each mesocosm received its corresponding dosage for each trial every three weeks for a 21-week period:
    - Mesocosm 1 (control): 0.94 mL of vegetable glycerin per trial
    - Mesocosm 2: 1 mL of nicotine solution per trial (60 mg/ trial)
    - Mesocosm 3: 10 mL of nicotine solution per trial (600 mg/ trial)
- All mesocosms were monitored multiple times each week for mortalities and temperature, pH, and dissolved oxygen were recorded.

Results

Trial Number	Mesocosm 1 (control)	Mesocosm 2 (1 mL/ trial)	Mesocosm 3 (10 mL/ trial)
1	0	0	20
2	0	0	--
3	0	0	--
4	0	0	--
5	0	0	--
6	0	0	--
7	0	1	--

Fig. 2- Table representing the number of mortalities in each mesocosm occurring within 24 hours of each trial.

Trial Number	Mesocosm 1 (control)	Mesocosm 2 (1 mL/ trial)	Mesocosm 3 (10 mL/ trial)
1	0	0	--
2	0	0	--
3	0	0	--
4	0	0	--
5	0	1	--
6	1	2	--
7	0	5	--

Fig. 3- Table representing the number of mortalities in each mesocosm occurring within 25-504 hours of each trial.

Fig. 5- All three mesocosm. Each mesocosm contained 200 L of water with 20 tadpoles.

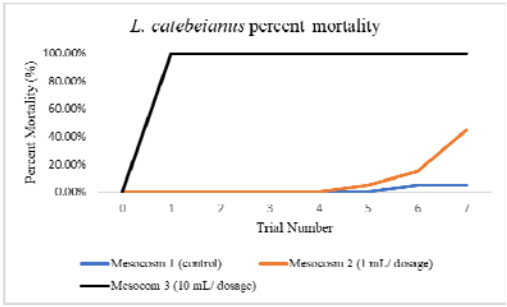

Fig. 4- The line graph shows the increasing percent mortality in the increasing amounts of nicotine

- There was a significant difference in mortality rate between the experimental groups and the control group.
- One mortality occurred in Mesocosm 1 (control), 9 mortalities in Mesocosm 2, and 20 mortalities in Mesocosm 3.
- There was a 100% mortality rate in Mesocosm 3, all within 24 hours of the dosage during Trial 1 (3 mg of nicotine/ L of water).
- Mortalities rose exponentially in mesocosm 2 after Trial 5 (1.5 mg of nicotine/ L of water).
- There was no significant difference between the Gosner stages of Mesocosm 1 (control) and Mesocosm 2 at the end of the 21 weeks.

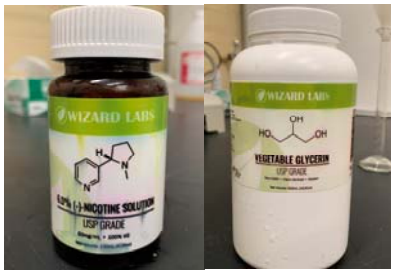

Fig. 6- A bottle of 6% nicotine and 94% VG (on the left) and a bottle of 100% VG (on the right) from Wizard Labs.

Discussion

- After analyzing the results, there is a direct correlation between increasing nicotine concentrations and increasing mortality rates.
- Most mortalities occurred more than 24 hours after each trial.
- The threshold nicotine level *L. catebeianus* seems to be between 1.2 – 1.5 mg of nicotine/ L of water.
- Future studies could determine the exact threshold or how *L. catebeianus* recovers from nicotine exposure
- This information shows how only a small amount of one chemical found in a cigarette butt can affect the aquatic environment.
- While smoking rates have dropped in the US, cigarette litter is still highly abundant. It is up to the public and government to put a stop to cigarette pollution.

Fig. 7- *L. catebeianus, or the American Bullfrog is an invasive species in Nebraska and acts as an indicator species to aquatic ecosystems.*

References and Acknowledgements

Acknowledgements:

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